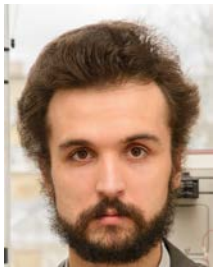
Clinical Chemistry

Thrombin-Mediated Degradation of Human Cardiac Troponin T



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Guest: Dr. Ivan Katrukha is a researcher at HyTest Limited in Turku, Finland, and a researcher in the Department of Biochemistry at the School of Biology of Moscow State University.

Bob Barrett: This is a podcast from *Clinical Chemistry* sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I'm Bob Barrett.

The differences between clinical laboratory analysis performed in serum and plasma are not always appreciated. Because serum is the liquid portion of the blood after it has been allowed to clot, it contains metabolites that result from the clotting process. Coagulation involves the activation of a number of proteases including thrombin. Thrombin is one of the most abundant proteases in serum and is capable of cleaving a large number of proteins forming degradation products. Immunoassays are designed to detect specific epitopes on proteins and may respond differently to degraded versus intact forms. Thus, knowing how pre-analytical processes and specific clinical conditions impact the stability of proteins is relevant for new assay development and in the investigation of questionable results from existing immunoassays. Mass spectrometry can be used to identify protease cleavage sites enabling increased understanding of the proteolytic degradation of clinically relevant molecules.

An original article appearing in the June 2017 issue of *Clinical Chemistry* uses this approach to investigate the stability of cardiac troponin T in patients with acute myocardial infarction. We are joined in this podcast by the article's first author, Dr. Ivan Katrukha. He is a researcher at HyTest Limited in Turku, Finland and is a researcher in the Department of Biochemistry at the School of Biology of Moscow State University.

So Doctor, what is the main idea of your study?

Dr. Katrukha: Well, our study is devoted to the degradation of cardiac troponin T and cardiac troponin T as one of the major, well-known biomarkers of acute myocardial infarction is measured in blood of patient with presumed heart attack by means of immunoanalysis. And the information about the stability of this biomarker is very important for the development of immunoassays if the degradation of the protein, the degradation side lies between the epitopes of

the antibodies that are used for this immunoanalysis in the immunoassay. The concentration of the analyte of the protein will be underestimated in the patient's samples.

So it was previously shown that troponin T is prone to degradation and a number of protein fragments were identified in patients' serum with the 29-Kilodalton fragments being a major one.

Bob Barrett: So Doctor, why is the study of troponin T degradation so important?

Dr. Katrukha: Now, the only one diagnostic system that is present in the market, it utilizes the antibodies that are specific to the central region of troponin T, but in the same time we might expect the appearance of new systems in the nearest future. And so the knowledge about the degradation of cardiac troponin T in blood or in serum or plasma that is used for analysis is crucial for the development of diagnostic system and forces for the selections of antibodies.

The knowledge of the degradation of cardiac troponin T in blood or in serum or in plasma that are usually used for the immunoanalysis is crucial both for the selection of the antibodies used in the immunoassays and for the selection of the matrix that would be used in the analysis.

Bob Barrett: So Doctor, let's get down to it, what are some of your findings?

Dr. Katrukha: As I have already said, the 29-Kilodalton fragment was found a major form of cardiac troponin T in serum samples and for a long time, they thought the intracellular protease calpain is one that is responsible for the cleavage of troponin T in necrotic myocardium and formation of this 29-Kilodalton fragment. But while studying the degradation of cardiac troponin T, we compared the protein that we purified from the serum samples and from heparin plasma samples of patients with myocardial infarction, and we saw that while in serum samples, troponin T is almost fully degraded, in heparin plasma samples, troponin is present mostly by the intact molecule, meaning that in circulating blood, troponin is also present as the intact molecule but not as a fragment. And we presume that the fragmentation of the protein, as seen by others in serum samples, takes place mainly during the preparation of serum samples.

In the series of experience we performed and described in our study, we show that the main enzyme that cleaves troponin T with the formation of 29-Kilodalton fragment is one of the most abundant enzymes of coagulation cascade, thrombin. Mass spectrum studies have shown that thrombin cleaves cardiac troponin T between amino acid residue 68

and 69 with a formation of these 29-Kilodalton fragments. At the same time, we see that some degradation of cardiac troponin T takes place in heparin plasma as well. We still are not sure about the cause of this degradation but we may presume that it may take place both in cardiac, myocardial or after the release of the proteins into the blood flow or both. We are not sure about what proteases cleave the protein and it's definitely the theme for the further investigation.

Bob Barrett: Finally Doctor, does this have any impact on any currently used assays?

Dr. Katrukha: Well, as I said before now in the market, there's only assay that is capable of measuring cardiac troponin cTnT assay utilizes monoclonal antibodies that are specific to the central part of the molecule. I haven't seen any studies that show how the degradation of cardiac troponin T with a formation of 29-Kilodalton fragments affect the performance of this only immunoassay. At the same time as I said already, we might expect the development of new immunoassays specific to the cardiac troponin T, and our study might be important for those scientists who will participate in developing those new assays.

Bob Barrett: Dr. Ivan Katrukha is a researcher at HyTest Limited in Turku, Finland, and a researcher in the Department of Biochemistry at the School of Biology of Moscow State University. He has been our guest in this podcast from *Clinical Chemistry*. I'm Bob Barrett. Thanks for listening.